Int. J. Med. Arom. Plants, ISSN 2249 – 4340
Vol. 2, No. 3, pp. 428-433, September 2012

RESEARCH ARTICLE

Determination of Oleuropein in leaves and fruits of some Syrian olive varieties

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Article History: Received 4th July 2012, Revised 1st August 2012, Accepted 2nd August 2012.

Abstract: Oleuropein contents (OLE) in leaves and fruits of from seven olive Syrian varieties harvested at spring and fall were determined by high-performance liquid chromatography. The results showed that, the concentration of OLE leaves samples measured in spring was higher than those determined in fall samples, where the OLE concentration in spring samples ranged between 5.6 - 9.2 mg/g. The highest concentration (9.2 mg/g dry leaves), was found in Jlott variety and the lowest concentration (5.6 mg/g dry leaves) was in Dan mawi. In fall olive leaves samples, the concentration of OLE ranged between 4.3 to 8.2 mg/g dry leaves, where the highest concentration was in Jlott variety and the lowest was in Dan mawi variety. As for fruit samples, the OLE concentration ranged between 635-1026 mg/kg, where the highest value was in Jlott variety, and the lowest was in Kaissy variety. Comparisons between olive oil content in fruits and oleuropein content in leaves (in spring and autumn) (using student T test statistical analysis) indicate that the differences in oleuropein contents are affected by the genetic makeup of the olive varieties and not by the environment.

Keywords: Oleuropein; *Olea. europaea*; leaf; fruit; HPLC.

Introduction

Olea europaea L. is a typical tree widely cultivated for oil production in the Mediterranean area. According to 2010 statistics, there are around 97 million olive trees in Syria covering 647.000 hectares (Ministry of Agriculture 2010). *Olea europaea* L. is widely studied for its alimentary use the fruits and the oil are important components in the daily diet of a large part of the world's population, whereas the leaves are important for their secondary metabolites such as the phenolic compounds (Hansen et al. 1996). Phenolic compounds present in *Olea europaea* fruits and leaves vary in qualitative and quantitative terms during the development and ripening process (Amiot et al. 1989). There is compelling scientific evidence that olive leaf polyphenols are bioactive compounds (De Leonardis et al. 2008). According to Petridis et al. (2012), the chemical composition of olives relies on some agronomical factors, one of which is the cultivar.

Olive leaf was first used medicinally in Ancient Egypt and was a symbol of heavenly power. More recent knowledge of the olive leaf's medicinal properties dates back to the early 1800s when pulverised leaves were used in a drink to lower fevers. A few decades later, green olive leaves were used in tea as a treatment for malaria (Somova et al. 2003). Since then, several researchers demonstrated antiviral (Micol et al. 2005), anti-HIV (Lee-Huang et al. 2003), antimicrobial (Bisignanoet al. 1999), antioxidant and anti-inflammatory (Mann et al. 1999; Briante et al. 2002), atherosclerosis inhibition and hypotensive (Somova et al. 2003; Khayyal et al. 2002), and anticarcinogenic properties that lead to the prevention of some cancers (Owen et al.2004), and finally, stimulation of the thyroid

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activities of olive leaf extract. (Al-Qarawi et al. 2002).

The leaves consist of secoiridoids (Skerget et al. 2005), phenolic compounds, flavonoids and essential oils (Altarejos et al. 2005). Oleuropein (OLE) is a secoiridoid, which is the most abundant phenolic compound in olive leaves and fruits and is responsible for the characteristic bitterness of olive fruit (Soler-Rivas et al. 2000; Andrews et al. 2003). OLE concentrations can reach up to 140 mg g^{-1} on a dry matter basis in young olives (Amiot 1986), and 60–90 mg g^{-1} of dry matter in the leaves (Khan et al. 2007). It has several pharmacological properties including antioxidant (Visioli et al. 2002), anti-inflammatory (Visioli et al. 1998), anti-atherogenic (Carluccio et al. 2003), anti-cancer (Owen et al. 2000), antimicrobial (Tripoli et al. 2005), and antiviral (Fredrickson et al. 2000), these reasons, OLE is commercially available as food supplement in Mediterranean countries. In addition, oleuropein has been shown to be cardio protective against acute adriamycin cardiotoxicity (Andreadou et al. 2007), and has been shown to exhibit anti-ischemic and hypolipidemic activities (Andreadou et al. 2006).

Several previous studies have reported oleuropein content of olive leaf and fruit, ethanolic and methanolic extracts (Lee-Huang et al. 2003; Benavente-Garcia et al. 2000; Bernard et al. 1992; Savournin et al. 2001; Altinyay and Altun 2006; Ansari et al. 2011; Arslan and Ozcan 2011; Esti et al. 1998).

The aim of this study was to determine OLE content in leaves and fruits of seven Syrian olive varieties to during spring and autumn seasons.

Materials and methods

Sample

Leaves of seven olive tree varieties (*Olea europaea* L.): Kaissy, Jlott, Sorani, Dan mawi, Khodeiri, Istanbuli and Nabali grown in South of Syria (Al- quneitra), were randomly collected in April and October 2011. Olive fruits were also randomly picked at optimum ripening stages for production of olive oil, according to their skin colour, in October 2011. Fresh leaves were dried in a ventilated oven for 72 h at 40 ºC., and grind to obtain olive leaves powder, which were stored at -20 until extraction.

Chemicals

Oleuropein (Sigma; 0889) used as the standard chemical was obtained from Sigma. Chromatographic grade-double distilled water, HPLC grade acetonitrile (Merck – 1.00029), and analytical grade formic acid (Sharlau; 98 - 100%) were used.

Standards solution

Stock solutions (100mg/ml Oleuropein / methanol). Diluted solutions were prepared in water: methanol (80:20, v/v) at 0.1, 0.5, 1, 2 mg/ml.

Extraction of Oleuropein

Leaves were extracted according to the methods of Altinyay and Altun (2006). Briefly, 5 g of the dried powdered materials were macerated with 50 ml methanol for 2h at room temperature using a magnetic stirrer. The extracts were filtered and evaporated to dryness under a temperature not exceeding 40 °C. The residues were dissolved in 50 ml of HPLC grade Merck methanol. Solutions were passed through a 0.45 µm filter and 20 µl extracts were directly injected into the HPLC.

Oleuropein was extracted from olive fruits using the method of Vinha et al. (2005) with modifications. Briefly, 1.5 g of sample were extracted with 20 ml of 80:20 (v/v) methanol-water. The mixture was homogenized using an Ika homogenizer, and then centrifuged at 3000 rev/min. for 5 min and the supernatant was filtered through filter paper, 2 ml were passed through a 0.45 µm filter and 20 µl extracts were directly injected into the HPLC. The results were obtained as a mean value of three separate injections.

HPLC-DAD system for analysis of Oleuropein

Chromatographic separation was achieved with LC system form Agilent (Infinity 1260) coupled with a diode array detector (DAD), using a reversed-phase Eclipse C18, (250 × 4.6, 3.5 µm) column from Agilent Co. the separation was conducted at 35°C. The mobile phase consisted of Water: Acetonitrile : Formic Acid (84.6 : 15 : 0.4). The flow rate was 1ml/min, and the injection volume 20 µl., with UV detection at 240 nm.

Statistical Analysis:

To test correlation between various parameters we used student´s *t- test* (SAS 1998). Histogram was generated using Excel program (Microsoft).

Results and Discussion

Oleuropein content in leaves and fruits of seven Syrian olive varieties is given in Table 1.

Table 1: Oleuropein content in leaves and fruits of some Syrian olive varieties.

Varieties	Spring samples	Fall Samples	
	OLE content in leaves samples (mg/g)	OLE content in leaves samples (mg/g)	OLE content in fruits samples (mg/kg)
Kaissy	7.4 ± 0.1	5.3 ± 0.4	634.6 ± 4.1
Jlott	9.2 ± 0.3	8.2 ± 0.3	1025.5 ± 5.8
Sorani	8.4 ± 0.4	6.5 ± 0.6	945.8 ± 6.1
Dan mawi	5.6 ± 0.4	4.3 ± 0.5	730.8 ± 6.6
Khodairi	6.1 ± 0.2	5.2 ± 0.4	680.4 ± 5.8
Istanbuli	8.1 ± 0.3	6.4 ± 0.4	985 ± 8.2
Nibaly	5.8 ± 0.3	4.5 ± 0.5	665.2 ± 5.8

As previously observed, the concentration of Oleuropein measured in spring olive leaves samples was higher than that determined in fall samples. OLE concentration in spring samples ranged between 5.6 - 9.2 mg / g dry leaves. The highest concentration of OLE was found in Jlott variety (9.2 mg / g dry leaves) and the lowest was found in Dan mawi variety (5.6 mg / g dry leaves). In the others varieties, concentration of OLE were 7.4, 8.4, 6.1, 8.1 and 5.8 mg / g dry leaves for Kaissy, Sorani, Khodeiri, Istanbuli and Nabaly varieties, respectively. In fall olive leaves samples, the concentration of OLE ranged between 4.3 to 8.2 mg /g dry leaves. The highest concentration was found in Jlott variety (8.2 mg / g dry leaves), whereas, the lowest concentration was in Dan mawi variety 4.3 mg /g dry leaves. The concentration of OLE in Kaissy, Sorani, Khodairi, Istanbuli and Nabaly varieties was 5.3, 6.5, 5.2, 6.4 and 4.5 mg/ g dry leaves, respectively. As for the fruit samples, the concentration of OLE ranged between 635 - 1026 mg/kg, where the highest concentration was in Jlott variety, and the lowest was in Kaissy variety. The concentration of OLE in the other varieties were 945.8, 730.8, 680.4, 985, 665.2 mg / kg., for Sorani, Dan mawi, Khodairi, Istanbuli and Nabaly, respectively.

Using olive oil data (GCSAR 2007) we have been able to compare content of oleuropein in leaves (spring and autumn) with content of olive oil in fruits (as shown in figure 1). As we can see there has been a high correlation between contents of oleuropein in leaves in spring and autumn ($p<0.0001$ using student T test). Also there has been a significant correlation between olive oil content and oleuropein (both in spring and autumn) ($p<0.0047$, $p<0.0026$ spring and autumn respectively). Finally, we compared contents of oleuropein in leaves and olive oil content in fruits (autumn) and also a high significant correlation ($p<0.0001$). These comparisons indicate that the differences in oleuropein contents are affected by the genetic makeup of the olive varieties and not by the environment.

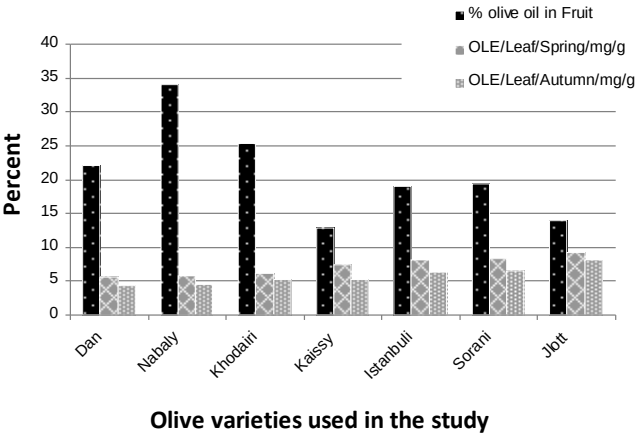

Figure 1: The correlation between contents of oleuropein in leaves and olive oil content in fruits

Comparing our results with those of other studies, concentrations of OLE in the Syrian varieties were relatively similar to those recorded

in the Iranian, Turkish, and Italian varieties. As indicated by Ansari et al. (2011), the OLE concentration in leaves of some Iranian olive varieties ranged between 6.1 - 13 mg / g dry leaves, and the highest concentration was in Shiraz variety 13 mg / g dry leaves. The OLE content in fruits of turkish olive variety "Sariulak" ranged between 644.2- 1222.7 mg/kg (Arslan and Ozcan 2011), while italian olive varieties contained 850 – 2080 mg OLE /kg (Estiet al.1998).

Conclusion

The highest concentration of OLE was found in fruits Jlott variety, followed by Istanbuli, Sorani, Dan, Khodeiri, Nabaly and Kaissy, respectively. While, in leaves was found in Jlott variety, Sorani, Istanbuli, Kaissy, Khodeiri, Nabaly, and Dan, respectively. Oleuropein contents in leaves less than the content of fruits. Oleuropein contents of leaves in spring, slightly higher than the content of the leaves in autumn.

Olive leave, is a cheap and wealth source of OLE. According to the results of this work one can select the varieties to be used for the preparation of medicines containing high levels of this important antioxidant.

Acknowledgement: The authors would like to express their thanks and gratitude to Professor Ibrahim Othman, Director General of Atomic Energy Commission of Syria and Professor Nizar Mir Ali for support and encouragement.

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